

## Use of the ectomycorrhizal fungus *Laccaria laccata* in forestry. I. Consistency between isolates in effective colonization of containerized conifer seedlings

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Four isolates of the ectomycorrhizal fungus *Laccaria laccata* (Scop. ex Fr.) Berk and Br. were inoculated singly onto containerized Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), Sitka spruce (*Picea sitchensis* (Bong.) Carr.), and western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) seedlings to detect ecotypic variation in colonization success and effects on seedling growth. All isolates formed well-developed ectomycorrhizae on all inoculated seedlings. Abundance ratings of short roots colonized did not differ between the four isolates for any conifer species; most inoculated seedlings developed ectomycorrhizae on over 80% of their short roots. There were also no differences between isolates in affecting seedling height, stem diameter, and dry weight of tops and roots for all conifer species. Inoculations did not improve seedling growth over uninoculated controls. Uninoculated controls of Douglas-fir and ponderosa pine had significantly greater dry weight than their inoculated seedlings; growth of Sitka spruce and western hemlock seedlings was not affected by any inoculation treatment. *Laccaria laccata* vigorously colonized the entire container rooting substrate and appears a prime candidate for artificial ectomycorrhizal inoculation of containerized western conifers.

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Quatre souches du champignon ectomycorhizateur *Laccaria laccata* (Scop. ex Fr.) Berk et Br. ont servi à inoculer, chacune séparément, des semis croissant en contenants, de Douglas taxifolié (*Pseudotsuga menziesii* (Mirb.) Franco), de pin à bois lourd (*Pinus ponderosa* Dougl. ex Laws.), d'épinette de sitka (*Picea sitchensis* (Bong.) Carr.) et de pruche occidentale (*Tsuga heterophylla* (Raf.) Sarg.), en vue de discerner une variation écotypique dans le succès de l'établissement du champignon et dans les effets sur la croissance des semis. Toutes les souches ont provoqué des ectomycorhizes bien développées chez tous les semis inoculés. Des mesures de l'abondance des racelles colonisées n'ont pas montré de différence entre les quatre souches sur chaque essence; la majorité des semis inoculés ont montré le développement d'ectomycorhizes sur plus de 80% de leurs racelles. On n'a pas non plus observé de différence attribuable aux souches pour ce qui est de la hauteur des semis, du diamètre de la tige et du poids sec des parties aériennes et des racines chez toutes les essences. L'inoculation n'a pas amélioré la croissance des semis par rapport aux témoins non-inoculés. Les témoins non-inoculés de Douglas taxifolié et de pin à bois lourd avaient un poids sec significativement plus élevé que les semis inoculés des deux mêmes espèces; les inoculations n'ont pas affecté la croissance des semis de l'épinette de sitka et de la pruche occidentale. *Laccaria laccata* s'est vigoureusement établi dans le système racinaire entier dans les contenants; il pourrait être un candidat à prioriser dans l'ectomycorhization artificielle par inoculation des conifères de l'ouest produits en contenants.

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### Introduction

Optimal nursery growth of containerized forest tree seedlings is often obtained through controlled greenhouse rearing, use of artificial potting substrates, and frequent applications of soluble fertilizers. Although seedlings reach plantable size in one growing season, roots typically lack natural ectomycorrhizal development or become sporadically colonized by such nursery-adapted fungi as *Thelephora* spp.

Efforts to inoculate containerized forest tree seedlings with selected ectomycorrhizal fungi have succeeded but with relatively few fungus species. Several species of containerized conifer seedlings have been

successfully inoculated with vegetative mycelium of *Pisolithus tinctorius* (Pers.) Coker and Couch (Marx and Barnett 1974; Ruehle and Marx 1977; Molina 1979; Marx *et al.* 1982) and improved outplanting performance of inoculated seedlings is particularly promising for regions in the southeast United States. *Cenococcum geophilum* Fr. and *Hebeloma crustuliniforme* (Bull. ex Saint-Amans) Quel. have similarly been used to inoculate several western United States conifers (Molina 1980; Shaw and Molina 1980; Shaw *et al.* 1982; J. M. Trappe and R. Molina, unpublished data). Another highly promising fungus in this regard is *Laccaria laccata* (Scop. ex Fr.) Berk. and Br. Vegetative mycelium of *L. laccata* has been highly effective in colonizing over 85% of the short roots on several containerized western conifers (Molina 1980). *Laccaria laccata* com-

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TABLE 1. Year of isolation, location, and associated hosts of four *Laccaria laccata* isolates used to inoculate containerized western conifers

Isolate	Year of isolation	Location and elevation	Associated hosts
S-238A*	1978	Benton Co., OR, 100 m	<i>Larix occidentalis</i>
S-283	1976	Union Co., OR, 1430 m	<i>Pseudotsuga menziesii</i> , <i>Pinus contorta</i>
S-326	1976	Benton Co., OR, 100 m	<i>Pseudotsuga menziesii</i>
S-444	1977	Benton Co., OR, 300 m	<i>Pseudotsuga menziesii</i>

\*Originally isolated in 1976 from Klamath Co., OR, at 1525-m elevation beneath *Tsuga mertensiana* (Bong.) Carr., but was reisolated from a sporocarp fruiting with an inoculated, containerized *Larix occidentalis* seedling (Molina 1980).

TABLE 2. Growth of containerized Douglas-fir seedlings inoculated with four isolates of *Laccaria laccata*\*

Isolate No.	Height, cm	Stem diameter, mm	Dry weight of tops, g	Dry weight of roots, g	Top/root
Control	18.74a	1.87a	0.62a	0.38a	1.65ab
S-238A	18.78a	1.68ab	0.44b	0.22b	2.06a
S-283	17.42a	1.74ab	0.47b	0.27ab	1.75ab
S-326	16.99a	1.62b	0.43b	0.27ab	1.64ab
S-444	16.06a	1.78ab	0.45b	0.24ab	1.43b

\*Means not sharing a common letter differ significantly ( $P \leq 0.05$ ) by Tukey test.

monly occurs in diverse forest habitats throughout the Pacific Northwest and other parts of the world; isolates are easily obtained from sporocarp tissue and grow well in culture.

Trappe (1977) emphasized the need for testing several strains or ecotypes of each fungus species before selecting an isolate for widescale use. Isolate variability in inoculum effectiveness demonstrated for *Pisolithus tinctorius* (Molina 1979; Marx 1981) reinforces this need. In this study, four isolates of *L. laccata* originating from diverse Oregon habitats were tested for their inoculum effectiveness and effect on container growth of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), ponderosa pine (*Pinus ponderosa* Dougl. ex Laws.), western hemlock (*Tsuga heterophylla* (Raf.) Sarg.), and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) seedlings.

### Methods

Four *Laccaria laccata* isolates from interior, high elevation forest types to low elevation coastal forests were used (Table 1). All were isolated from sporocarp tissue.

Mycelial inoculum in a vermiculite carrier was prepared according to Marx and Bryan (1975) as modified by Molina (1979, 1980). Inoculum of each isolate was grown aseptically

in glass-capped 2-L flasks containing 1450 mL of vermiculite plus 50 mL of sphagnum peat moss moistened with 750 mL of modified Melin-Norkrans nutrient solution (Marx 1969); dextrose was substituted for sucrose in this solution. Control flasks contained no fungus. After 3 months of incubation at room temperature, inoculum was removed from the flasks and leached with cold running tap water to remove unused nutrients. Excess free water was removed by gently squeezing the inoculum wrapped in cheesecloth. Inoculum was placed in plastic bags and stored overnight at 5°C.

A potting substrate containing equal volumes of sphagnum and vermiculite was steam pasteurized at 80°C for 30 min. Inoculum of each isolate and control inoculum was thoroughly mixed with the potting substrate (one part inoculum: six parts substrate) in large plastic bags and then used to fill individual "Ray Leach" containers (65-cm<sup>3</sup> capacity). A separate experiment and analysis were conducted for each of the four tree species. Each experiment consisted of a completely randomized block design with three replicate blocks and five fungus treatments (four fungus isolates plus one control of no fungus addition); 40 seedlings were grown per fungus treatment. This resulted in a total of 600 (3 × 5 × 40) seedlings in each experiment. Cells were sown with two or three stratified seeds of the appropriate conifer species. All were mist irrigated twice daily until seed germination was complete and then thinned to one germinant per cell.

Seedlings were greenhouse reared from mid-May through

TABLE 3. Growth of containerized Ponderosa pine seedlings inoculated with four isolates of *Laccaria laccata*\*

Isolate No.	Height, cm	Stem diameter, mm	Dry weight of tops, g	Dry weight of roots, g	Top/root
Control	12.13a	2.09a	0.48a	0.38a	1.28a
S-238A	9.55ab	1.94ab	0.35b	0.29b	1.28a
S-283	9.38b	1.96ab	0.34b	0.27b	1.32a
S-326	8.98b	1.78b	0.33b	0.24b	1.40a
S-444	9.75ab	1.95ab	0.39b	0.27b	1.48a

\*Means not sharing a common letter differ significantly ( $P \leq 0.05$ ) by Tukey tests.

October 1979. Supplemental light of approximately 11 000 lx set for a 15-h photoperiod was provided by overhead sodium-vapor lamps. Two weeks after germination, seedlings were fertilized biweekly through September with a soluble 20-19-18 N-P-K (Peter's Peat-lite special) fertilizer plus Sequestrene 300 iron chelate, both dissolved in tap water. At 2, 4, and 6 weeks all seedlings were hand watered to saturation with a solution containing 600 mg/L soluble fertilizer plus 300 mg/L iron chelate. All subsequent fertilizations were with twice the concentration of soluble fertilizer. Seedlings were mist irrigated with tap water three times weekly throughout the experiment.

Eight weeks after sowing, five seedlings from each treatment replication were examined for initiation of ectomycorrhizae. At the end of October, all remaining seedlings were harvested, and their roots washed free of substrate. Ten randomly selected seedlings from each treatment replication were examined for degree of ectomycorrhizal formation and measured for stem height and diameter and oven-dry weight of tops and roots. Degree of mycorrhizal formation was assessed by visually estimating the percentage of total short roots colonized by six categories: 0, no mycorrhizae; 1, 1-20% mycorrhizae; 2, 21-40%; 3, 41-60%; 4, 61-80%; 5, 80% or more. Roots of all uninoculated control seedlings were examined, and those with any detectable ectomycorrhizae were discarded. Thus, only control seedlings with no mycorrhizae were measured for growth comparison. This was necessary as some controls were contaminated with *Thelephora americana* Lloyd. All response variables, stem height and diameter, oven-dry weight of tops and roots, ratio of top dry weight to root dry weight, and percent of short roots colonized were subjected to analysis of variance; and significant differences between treatment means were separated by Tukey tests at  $P \leq 0.05$ .

### Results

All *L. laccata* isolates produced prolific ectomycorrhizal development on all inoculated seedlings of all conifer species. Eight weeks after sowing, sampled roots showed well-developed ectomycorrhizae; newly emerging, nub-like short roots had mantles and Hartig nets were easily detected microscopically. Differences between isolates in their rate of root colonization were not detected.

Ectomycorrhiza morphology was as described by

Molina (1980). Douglas-fir ectomycorrhizae developed into prolific pinnate and variously branched, elongate forms. Ponderosa pine ectomycorrhizae were initially simple to dichotomous, ultimately often becoming large compound branched coralloid clusters, especially on laterals just below the root collar. Sitka spruce and western hemlock ectomycorrhizae were primarily simple to variously branched but notably less elongate or highly branched than on Douglas-fir. Macroscopically, the smooth, pale orange-brown mycorrhizae with occasional lavender tints were indistinguishable between isolates. Control seedlings, however, lacked *L. laccata* mycorrhizae, so cross inoculation between the various isolates is unlikely.

Total ectomycorrhizal short roots did not differ between any inoculation treatments regardless of fungus isolate or host species. All but two inoculated seedlings had 80-100% of total short roots ectomycorrhizal. On many inoculated seedlings, nonmycorrhizal short roots were seldom seen. When seedlings were removed from the plastic containers, the densely penetrating mycelium held the root-substrate plug intact; abundant sporocarp primordia were noted along various sections of plug surfaces. From September through October, fully developed sporocarps were produced prolifically by all isolates on the four host species. Seedlings were examined daily during this time, and developing sporocarps were removed to avoid cross contaminations. Sporocarps were noted earliest on ponderosa pine and Douglas-fir and later on Sitka spruce and western hemlock.

Noninoculated control seedlings were mostly free of mycorrhizal development; approximately 3% were colonized by *Thelephora americana*. Nonmycorrhizal short roots were notably unbranched and had abundant root hairs; root hairs were especially dense on Sitka spruce.

Few significant differences appeared between *L. laccata* isolates regarding effects on seedling growth. Most differences occurred between controls and inoculated treatments. These effects are described by individual conifer species.

TABLE 4. Growth of containerized western hemlock seedlings inoculated with four isolates of *Laccaria laccata*\*

Isolate No.	Height, cm	Stem diameter, mm	Dry weight of tops, g	Dry weight of roots, g	Top/root
Control	15.81a	1.72a	0.37a	0.21a	1.82b
S-238A	17.09a	1.67a	0.39a	0.16a	2.42ab
S-283	17.91a	1.83a	0.41a	0.16a	2.76a
S-326	16.83a	1.82a	0.36a	0.17a	2.29b
S-444	16.79a	1.74a	0.35a	0.14a	2.74a

\*Means not sharing a common letter differ significantly ( $P \leq 0.05$ ) by Tukey tests.TABLE 5. Growth of containerized Sitka spruce seedlings inoculated with four isolates of *Laccaria laccata*\*

Isolate No.	Height, cm	Stem diameter, mm	Dry weight of tops, g	Dry weight of roots, g	Top/root
Control	17.64a	1.87a	0.46a	0.19a	2.73a
S-238A	16.62a	1.79a	0.43a	0.17a	2.63a
S-283	17.51a	1.88a	0.44a	0.17a	2.63a
S-326	17.47a	1.84a	0.43a	0.21a	2.12a
S-444	18.01a	1.83a	0.48a	0.22a	2.33a

\*Means sharing a common letter do not differ significantly ( $P \leq 0.05$ ) by Tukey test.*Douglas-fir*

Significant differences occurred between control and certain inoculation treatments for all growth measurements except height and top:root ratio (Table 2). Control seedlings had significantly greater stem diameters than seedlings inoculated with isolate S-326, greater dry weight of tops than seedlings inoculated with any of the four isolates, and greater root dry weight than seedlings inoculated with S-238A (Table 2). Only top:root ratio differed significantly between seedlings inoculated with certain isolates; isolate S-238A produced a significantly greater top:root ratio than isolate S-444.

*Ponderosa pine*

Mean seedling heights, stem diameters, and dry weights of tops and roots differed significantly between certain inoculation treatments and controls (Table 3). Control seedlings had significantly greater height than seedlings inoculated with isolates S-283 and S-326, greater stem diameter than seedlings inoculated with S-326, and greater dry weight of tops and roots over seedlings inoculated singly with any isolate. The four *L. laccata* isolates did not differ significantly for any growth variables.

*Western hemlock*

Significant differences between treatment means occurred only for certain top:root ratios; seedlings inoculated with isolates S-283 and S-444 had greater

top:root ratios than control seedlings or those inoculated with S-326 (Table 4).

*Sitka spruce*

No significant differences occurred between any inoculation and control treatments (Table 5).

**Discussion**

Ecotypes of ectomycorrhizal fungus species can vary in *in vitro* cultural characteristics, growth rates, temperature optima, and tolerance to extremes, nutrient uptake, and host response to inoculation (Trappe 1977). Various isolates of *Pisolithus tinctorius* differed significantly in their ability to colonize roots of several ectomycorrhizal host seedlings following mycelial inoculations (Molina 1979; Marx 1981). It was thus unexpected, in this study, to find no differences between the four *L. laccata* isolates in abundance of short roots colonized, regardless of host species. Nearly all inoculated seedlings received the highest abundance rating, and, indeed, most root systems were completely colonized. As seen in previous studies (Molina 1980), *L. laccata* appears well suited to the growing conditions of containerized seedlings; newly emerging short roots of young seedlings are quickly colonized. By season's end, the mycelium completely permeates the rooting substrate and sporocarps are produced in many cells.

Isolates did not differ in effects on seedling height,

stem diameter, or dry weight of tops and roots for any conifer species. Only top:root ratios of Douglas-fir and western hemlock differed significantly among certain isolate inoculations.

*Laccaria laccata* did significantly affect seedling growth, however, compared with noninoculated controls, especially on Douglas-fir and ponderosa pine seedlings; noninoculated seedlings had significantly greater top and root dry weights than all inoculation treatments for both conifers. Control seedlings of ponderosa pine were also significantly taller than those inoculated with isolates S-283 and S-326. Yet, for Sitka spruce and western hemlock, these same growth parameters were not affected significantly by any isolate inoculation. Thus, at least with *L. laccata*, seedling growth response following inoculation appears related to host species. This may be due in part to different host allocations of photosynthates from needles to roots as reflected by the large differences in top to root ratios of Douglas-fir and ponderosa pine versus Sitka spruce and western hemlock seedlings (Tables 2–5).

As seen in several recent studies (Marx and Barnett 1974; Ruehle and Marx 1977; Molina 1979, 1980; Shaw and Molina 1980), ectomycorrhizal inoculation rarely improves growth of containerized seedlings in the greenhouse, especially under the reduced fertility schedules used to maximize ectomycorrhizal development. Noninoculated seedlings are often larger than inoculated seedlings. This difference is attributed to fungus utilization of host photosynthates. With low fertility, confined rooting volume, and a highly active fungal component, including sporocarp formation, the drain on host photosynthate is considerable. Douglas-fir and ponderosa pine were more affected in this regard than either western hemlock or Sitka spruce. Encouragingly, however, *L. laccata* will colonize roots abundantly at higher fertility regimes to produce strongly mycorrhizal seedlings of plantable size (R. Molina and J. Chamard, unpublished data).

*Laccaria laccata* appears to be a leading candidate for artificial ectomycorrhizal inoculation of containerized nursery stock. Vegetative mycelium in a vermiculite carrier is highly effective inoculum as indicated by colonization of all inoculated seedlings. The fungus is well adapted to the growing conditions of containerized seedlings and highly active on the root system; maximum mycorrhizal development is consistently obtained. The consistency between isolates in colonizing ability strengthens the possible use of *L. laccata* in inoculation programs. Given the widespread occur-

rence of *L. laccata* over diverse forest habitats, its ease of isolation, and inoculum effectiveness, obtaining and selecting isolates specifically for improving outplanting performance of seedlings on a wide variety of sites is feasible.

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MARX, D. H. 1969. The influence of ectotrophic mycorrhizal fungi on the resistance of pine roots to pathogenic infections. I. Antagonism of mycorrhizal fungi to root pathogenic fungi and soil bacteria. *Phytopathology*, **59**: 153–163.

———. 1981. Variability in isolates of *Pisolithus tinctorius* as affected by source, age, and revitalization. *Can. J. For. Res.* **11**: 168–174.

MARX, D. H., and J. P. BARNETT. 1974. Mycorrhizae and containerized forest seedlings. In *Proceedings North American containerized forest tree seedling symposium*. Edited by R. W. Tinus, W. I. Stein, and W. E. Balmer. Great Plains Agric. Counc. Publ. **68**: 85–92.

MARX, D. H., and W. C. BRYAN. 1975. Growth and ectomycorrhizal development of loblolly pine seedlings in fumigated soil infested with the fungal symbiont *Pisolithus tinctorius*. *For. Sci.* **21**: 245–254.

MARX, D. H., J. L. RUEHLE, D. S. KENNY, C. E. CORDELL, J. W. RIFFLE, R. J. MOLINA, W. H. PAWUK, S. NAVRATIL, R. W. TINUS, and O. C. GOODWIN. 1982. Development of *Pisolithus tinctorius* ectomycorrhizae on containerized tree seedlings with vegetative inocula produced by commercial and research procedures. *For. Sci.* In press.

MOLINA, R. 1979. Ectomycorrhizal inoculation of containerized Douglas-fir and lodgepole pine seedlings with six isolates of *Pisolithus tinctorius*. *For. Sci.* **25**: 585–590.

———. 1980. Ectomycorrhizal inoculation of containerized western conifer seedlings. U.S. Dep. Agric. For. Serv. Res. Note PNW-357.

RUEHLE, J. L., and D. H. MARX. 1977. Developing ectomycorrhizae on containerized pine seedlings. U.S. Dep. Agric. For. Serv. Res. Note SE-242.

SHAW, C. G., III, and R. MOLINA. 1980. Formation of ectomycorrhizae following inoculation of containerized Sitka spruce seedlings. U.S. Dep. Agric. For. Serv. Res. Note PNW-351.

SHAW, C. G., III, R. MOLINA, and J. WALDEN. 1982. Development of ectomycorrhizae following inoculation of containerized Sitka and white spruce seedlings. *Can. J. For. Res.* **12**.

TRAPPE, J. M. 1977. Selection of fungi for ectomycorrhizal inoculation in nurseries. *Annu. Rev. Phytopathol.* **15**: 203–222.